

Immunogenicity and Efficacy of Polysaccharide and Polysaccharide-Protein Conjugate Vaccines in Neonates

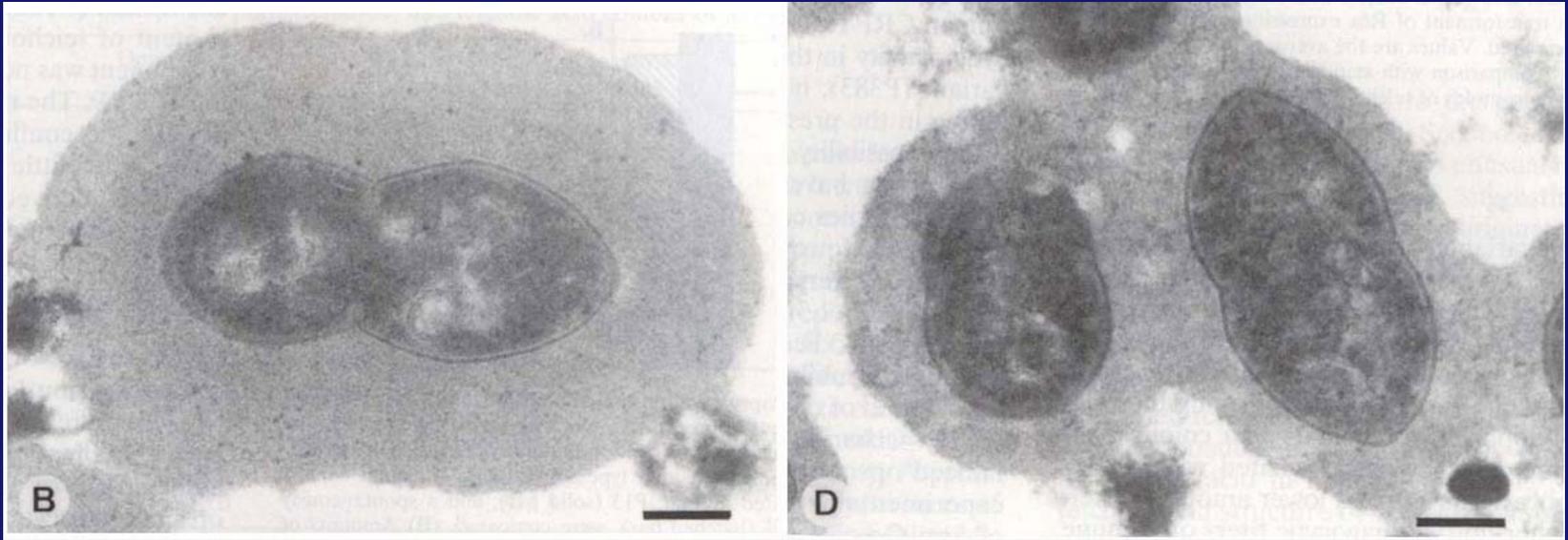
Session 4

The Antibody Response to Capsular Polysaccharide Antigens

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Encapsulated Pneumococci



J.O. Kim et al., Infection and Immunity, May 1999

THEMES

- **V Gene Repertoire**
- **Affinity/Avidity**
- **Molecular Basis of Antibody Function**
- **Ontogeny**

HAEMOPHILUS INFLUENZAE TYPE B (HIB): A MODEL HUMAN ANTI-POLYSACCHARIDE ANTIBODY RESPONSE

- ◆ **Encapsulated: 3- β -D-ribose-(1 \rightarrow 1)-D-ribitol-5-phosphate**
- ◆ **Pathogenic: meningitis, epiglottitis, septic arthritis**
- ◆ **Anti-Capsular Antibody Response**
 - **Protective**
 - **Ontogenically Regulated**
 - **Pauciclonal**
 - **Limited V Region Diversity**
- ◆ **Conjugate Vaccines**

V Region Gene Segments Encoding Anti-Hib PS Antibodies

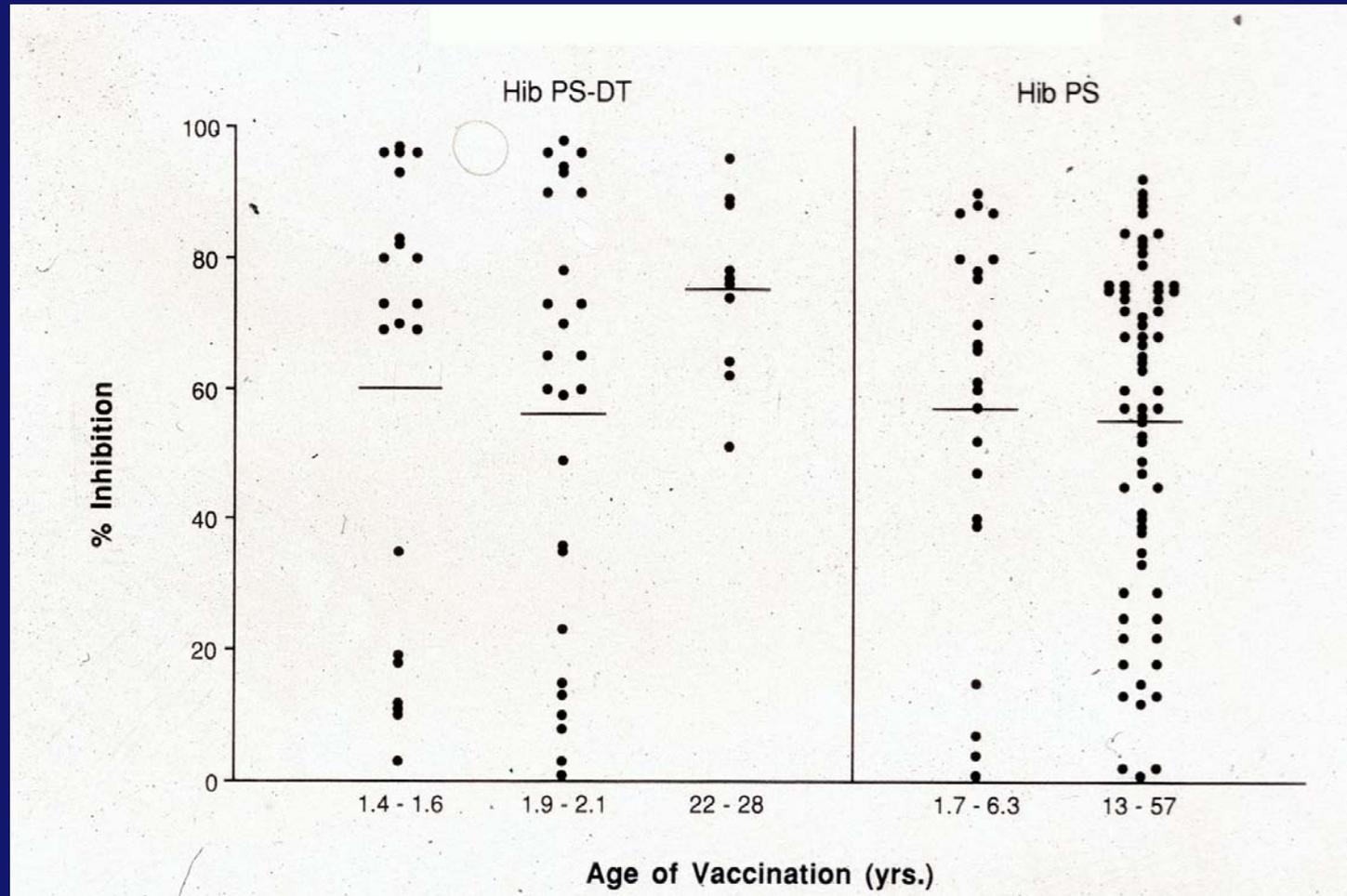
	VH		VL	Serological Probe
IIIa	V3-23	κI	08/018	
	V3-21		L11	
IIIb	V3-15		15A	
	V3-49	κII	A2	HibId-1
			A1/A17	
			A3/A19	
		κIII	A27	κIII
			L16	
		κIV	B3	
		λII	2.1	
		λIII	3.1	
		λVII	4A	HibId-2

Canonical Anti-Hib PS Combining Site

V3-23	Gly-Tyr-Gly-Met/Phe-Asp	JH4/6
V κ -A2	Arg (95a)	J κ 1/3

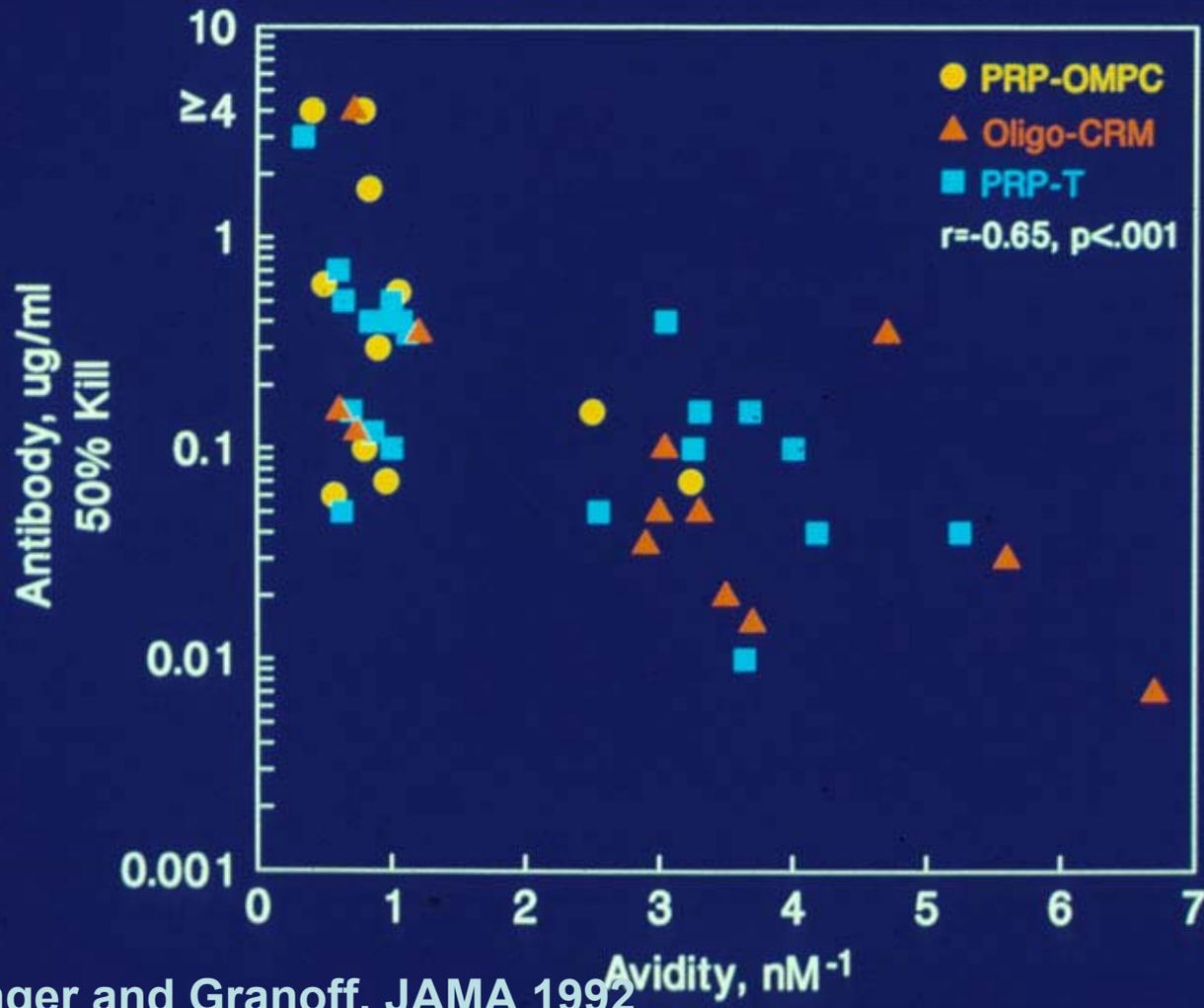
Hibd-1 Positive

Hibd-1 Expression After Vaccination



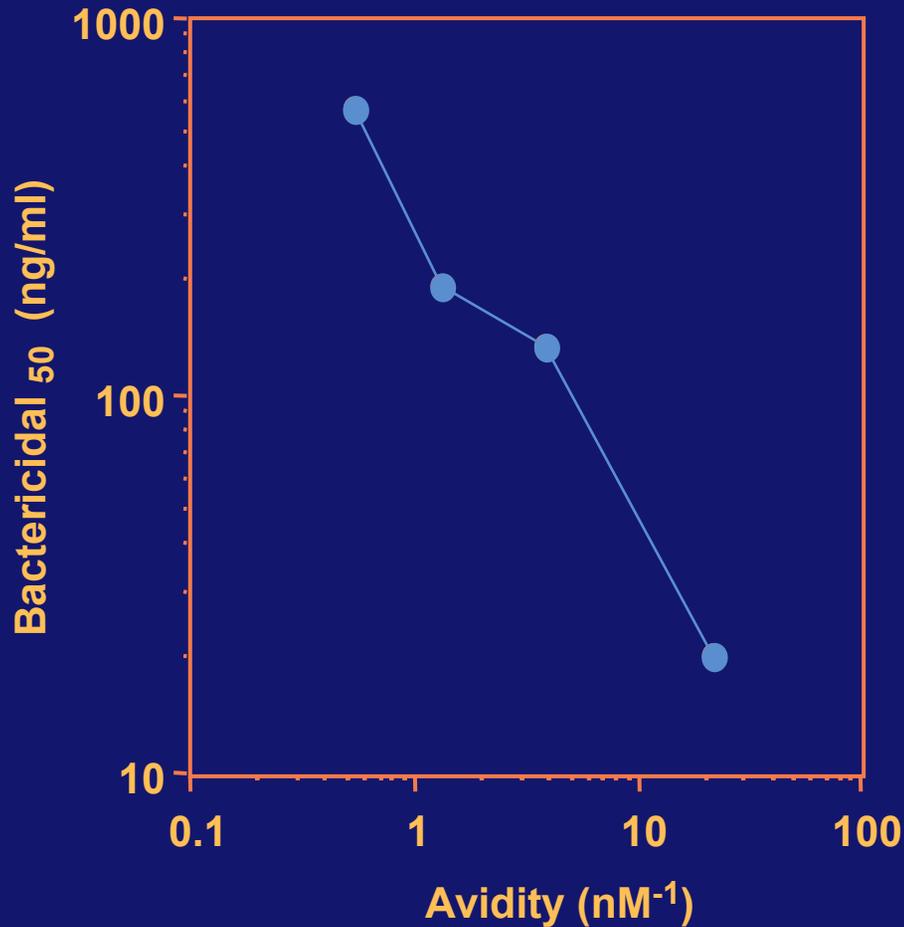
Lucas et al. J. Clin. Invest.88:1811 (1991)

Bactericidal Activity as a Function of Antibody Avidity



Schlesinger and Granoff, JAMA 1992

Correlation Between Hib PS Avidity and Bactericidal Activity



IgG1 Hib1d-1 Anti-Hib PS Antibodies Elicited by two Conjugate Vaccines

Vaccine	Avidity $\text{nM}^{-1} \pm \text{SE}$	Bactericidal $\mu\text{g/ml} \pm \text{SE}$	Protective Dose $\mu\text{g/rat}$
HbOC	4.36 ± 0.21	0.02 ± 0.02	≤ 0.06
Hib PS-OMPC	2.07 ± 0.17	0.27 ± 0.03	~ 1.0
P (t-test)	$< .01$	$< .002$	$< .002$

Lucas and Granoff. J. Immunol. 154:4195 (1995).

Mechanisms Generating Antibody Functional Diversity

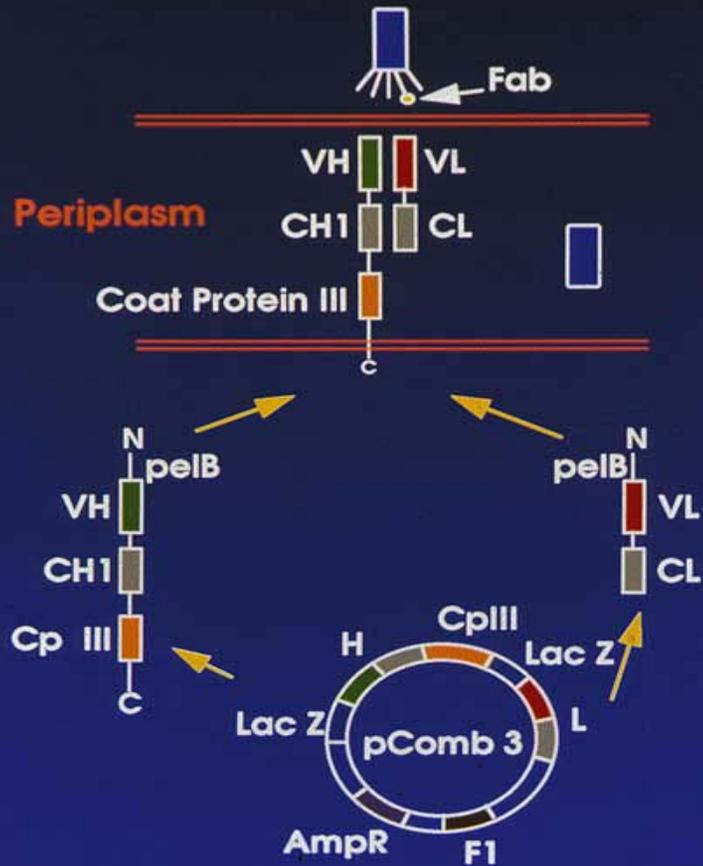
Inherited (germline)

- Individual differences in content of functional V genes
- V gene alleles / homologues differ in their specificity potential
- J κ segments differ in their specificity potential

Acquired (somatic)

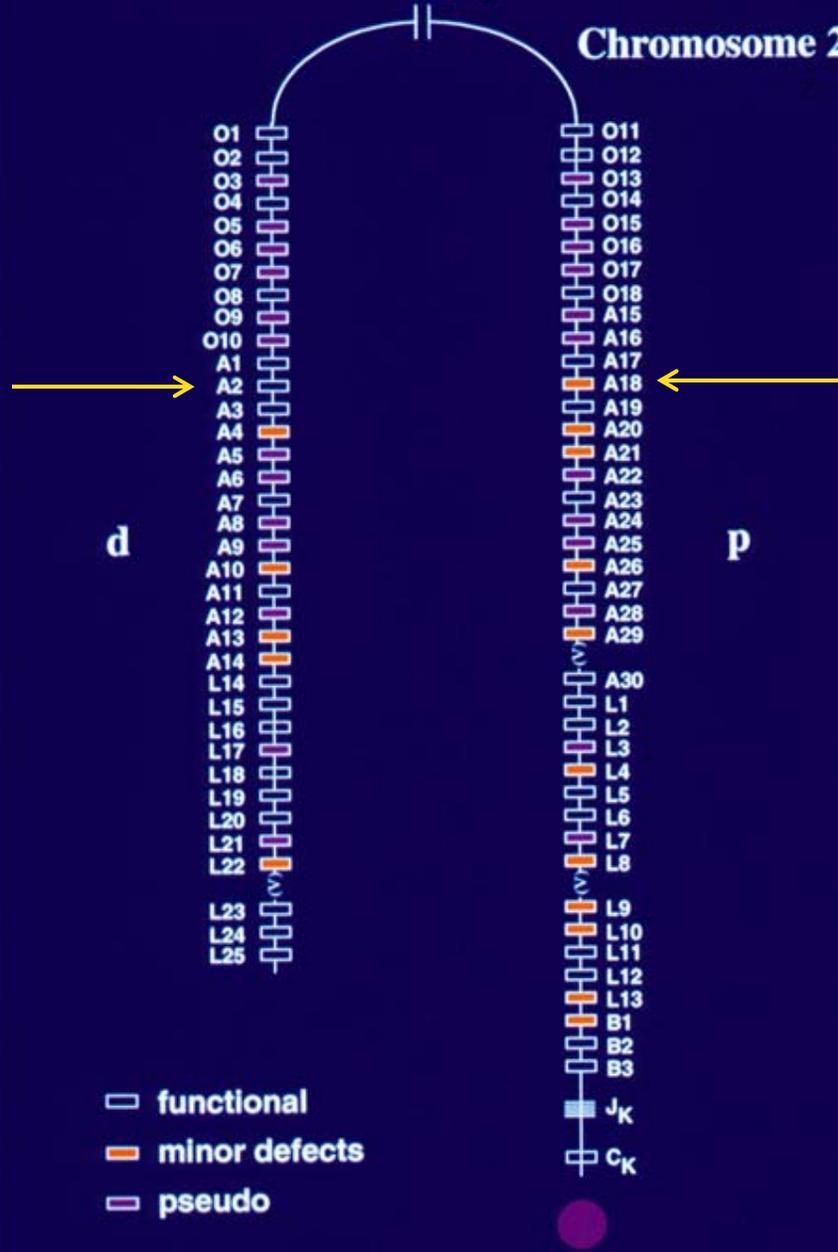
- V region pairing configurations
- Generation of CDR 3 during gene assembly
- Hypermutation: positive and negative impact
- Antigen (vaccine) selection

THE pCOMB 3 SYSTEM

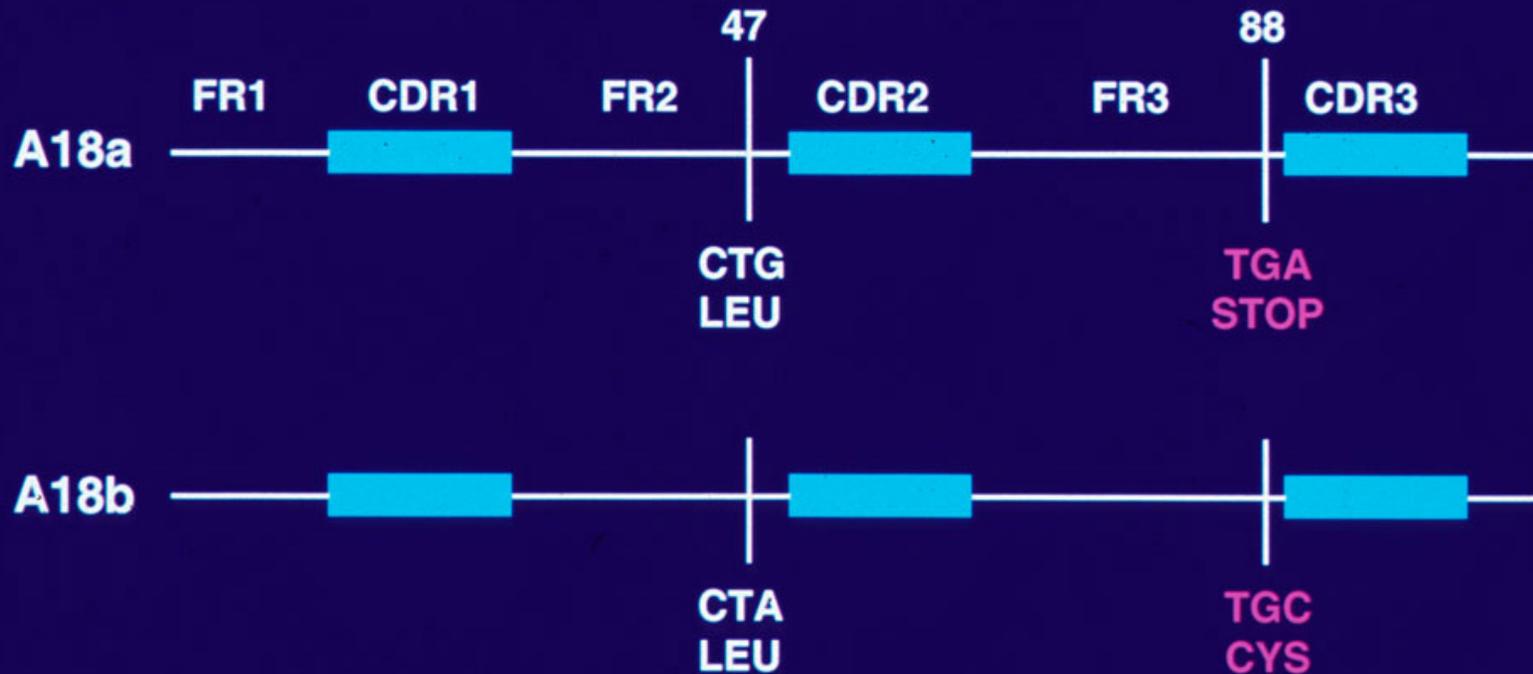


Human Kappa Locus

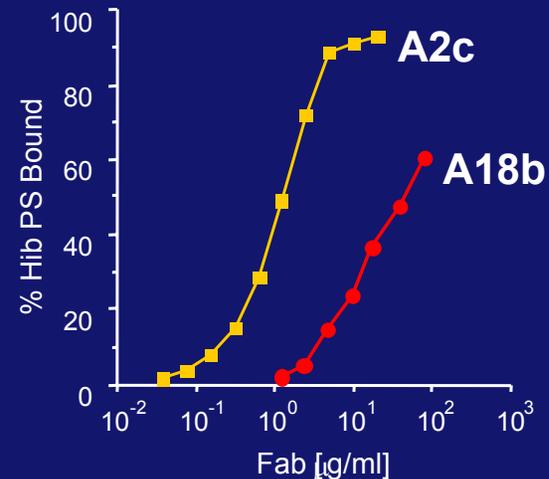
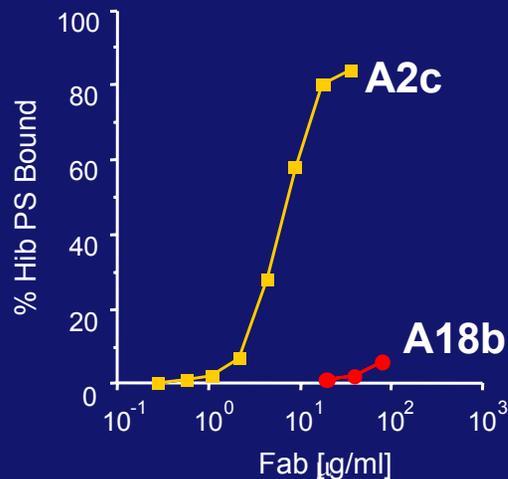
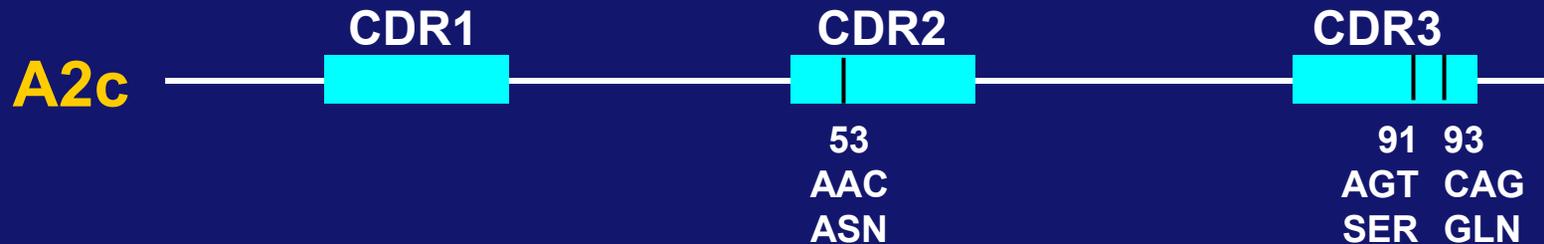
Chromosome 2



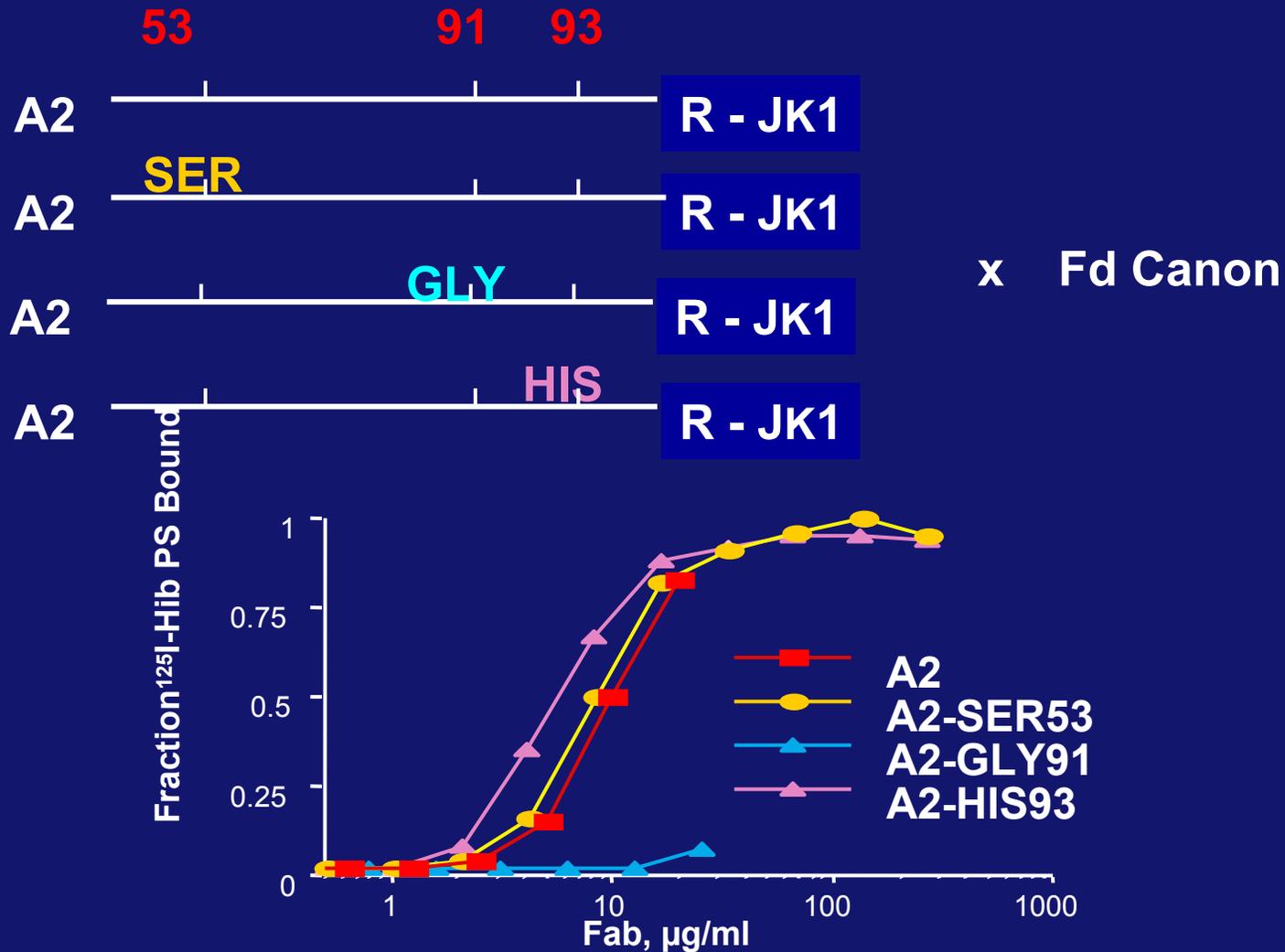
A Point Mutation Converts a Non-Functional VK Gene into a Functional Gene



VK Homologues Differ in their Potential to Form a Hib PS Combining Site



A Single Residue Accounts for the Different Binding Potential of A2c and A18b



Nucleotide Sequence of V3-23 and 3.7Kb Allele (Canonical Hib PS Configuration)

V3-23 GAG GTG CAG CTG CTC GAG TCT GGG GGA GGC TTG GTA CAG CCT GGG GGG TCC CTG AGA CTC TCC TGT GCA
3.7Kb -----

CDR-I

V3-23 GCC TCT GGA TTC ACC TTT AGC AGC TAT GCC ATG AGC TGG GTC CGC CAG GCT CCA GGG AAG GGG CTG GAG
3.7Kb -----

CDR-II

V3-23 TGG GTC TCA GCT ATT AGT GGT AGT GGT GGT AGC ACA TAC TAC GCA GAC TCC GTG AAG GGC CGG TTC ACC
3.7Kb -----T---TA-ACG---A-----T-----

V3-23 ATC TCC AGA GAC AAT TCC AAG AAC ACG CTG TAT CTG CAA ATG AAC AGC CTG AGA GCC GAG GAC ACG GCC
3.7Kb -----T-----

CDR-III

JH6

V3-23 GTA TAT TAC TGT GCG AAA GGC TAC GGT ATG GAC GTC TGG GGC CAA GGG ACC ACG GTC ACC GTC TCC TCA
3.7Kb -----

Amino Acid Sequence of V3-23 and 3.7Kb Allele Canonical Hib PS Configuration)

V3-23Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala
3.7Kb-----

CDR-I

V3-23Ala Ser Gly Phe Thr Phe Ser Ser Tyr Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu
3.7Kb-----

CDR-II

V3-23Trp Val Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr
3.7Kb----- Val --- Tyr Ser Gly --- Ser -----

V3-23Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala
3.7Kb-----

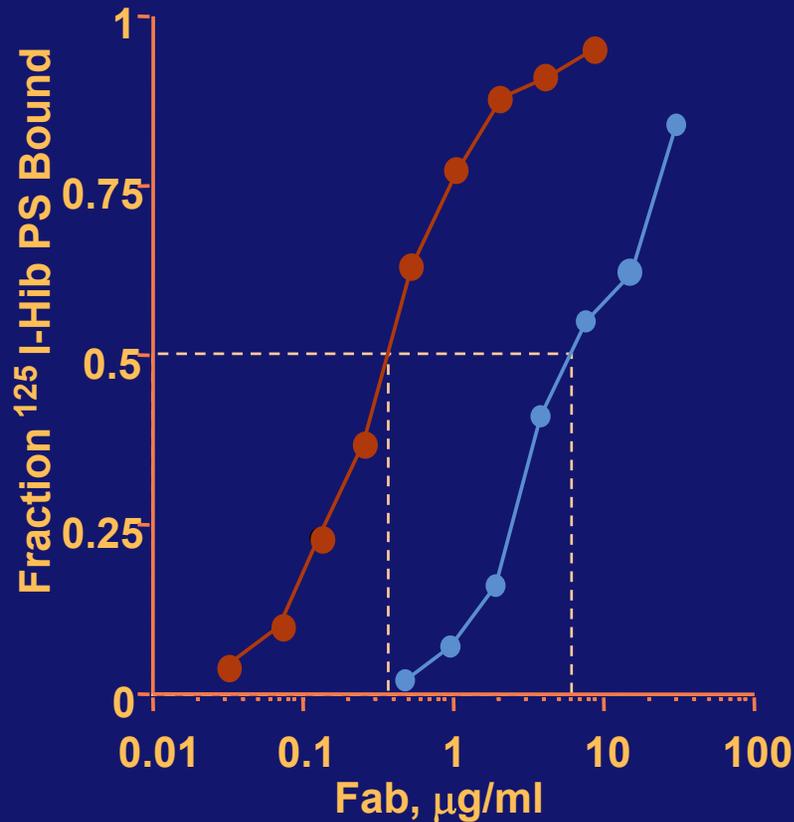
CDR-III

JH6

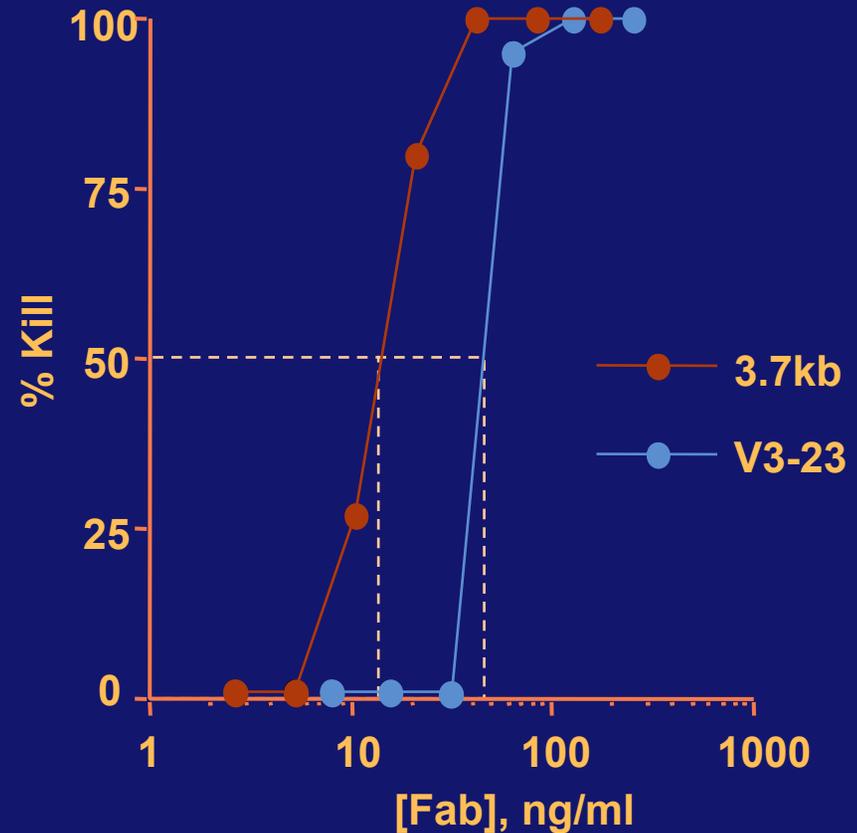
V3-23Val Tyr Tyr Cys Ala Lys Gly Tyr Gly Met Asp Val Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
3.7Kb-----

The 3.7 kb Allele Confers Higher Avidity and Bactericidal Activity

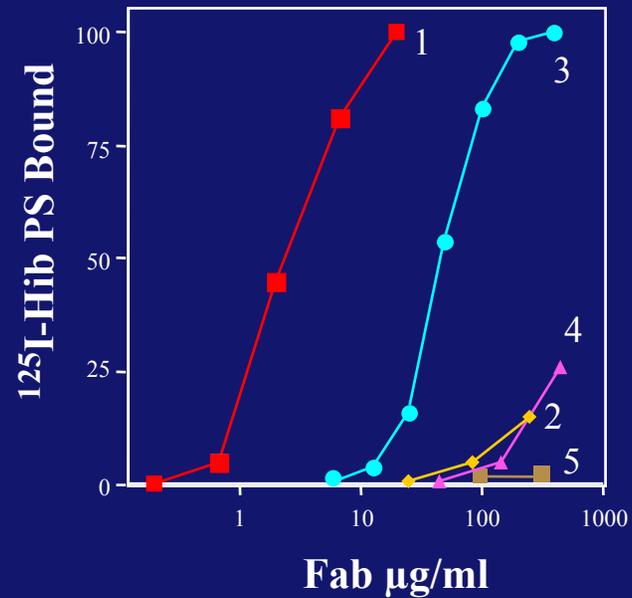
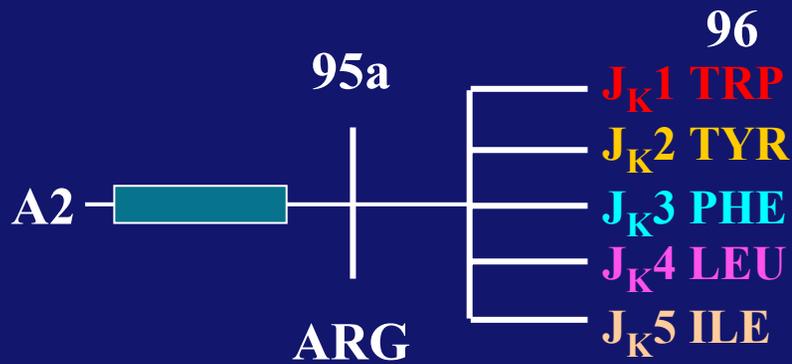
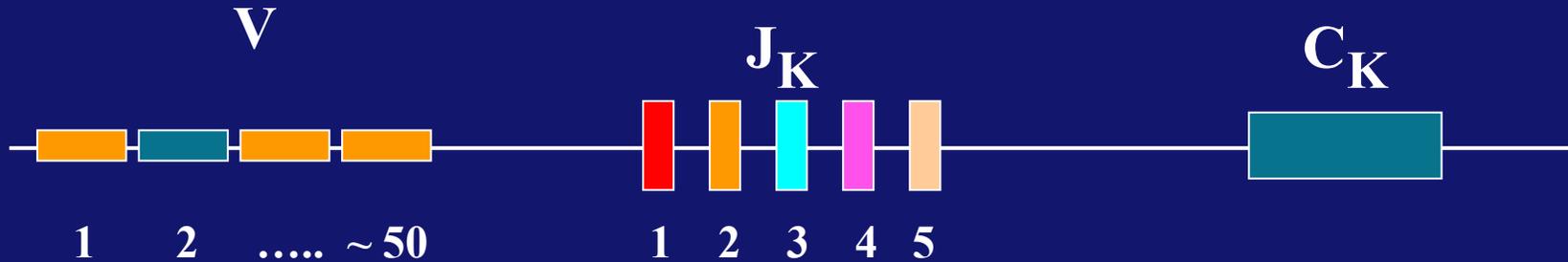
Radioantigen Binding



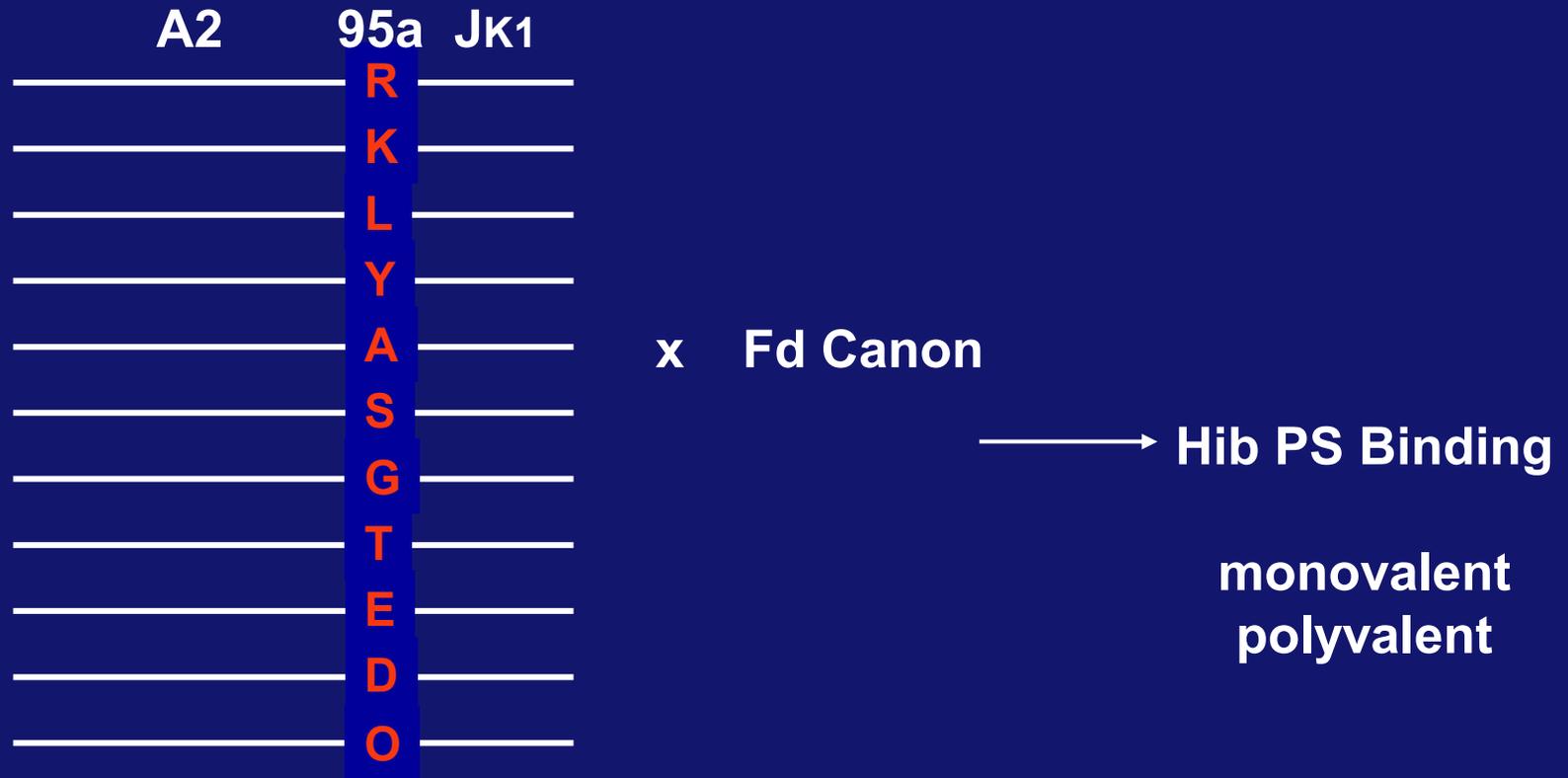
Bactericidal Activity



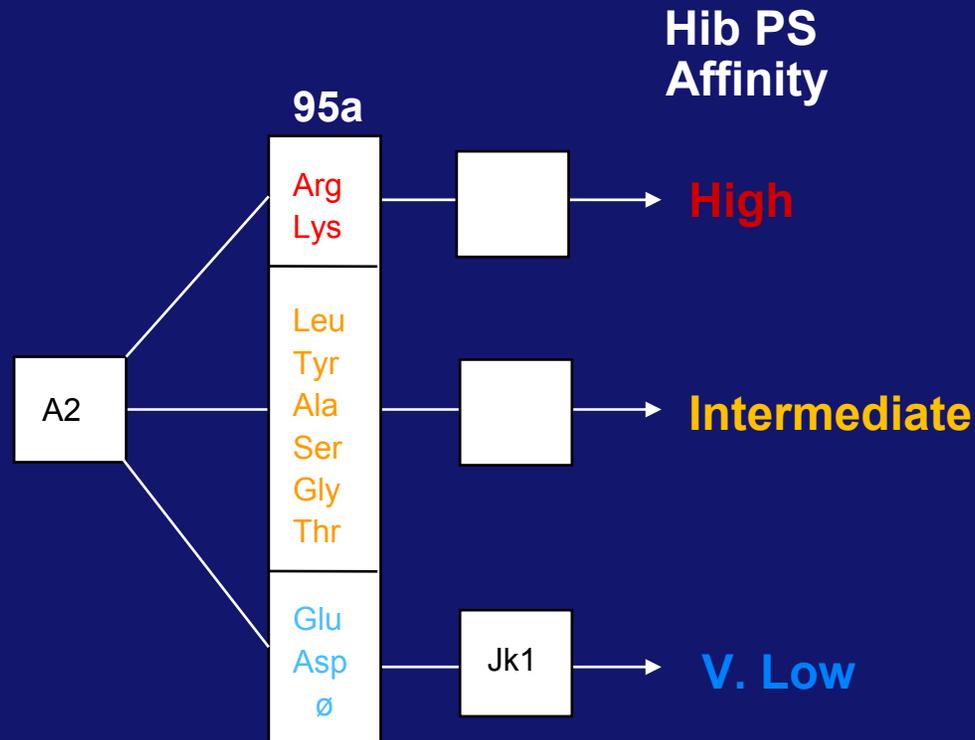
Effect of J_K Region on Hib PS Binding



Strategy for Evaluating the Role of L Chain Junctional Residue (95a) in Hib PS Binding



Junctional Amino Acid Determines Binding Affinity



Mechanisms Generating Antibody Functional Diversity

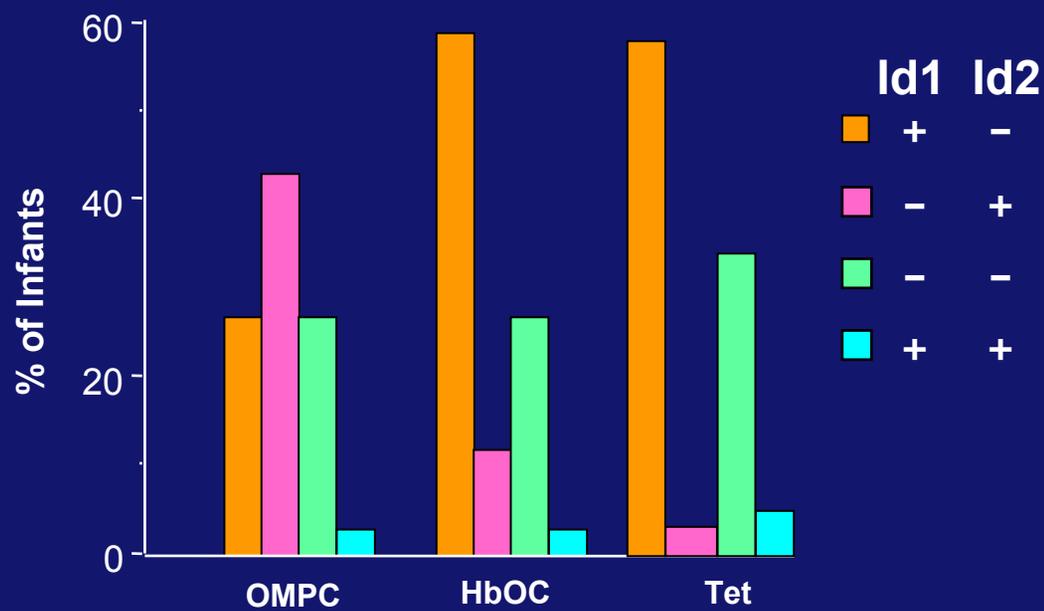
Inherited (germline)

- Individual differences in content of functional V genes: A18 can be a functional gene
- V gene alleles / homologues differ in their specificity potential: A2 vs A18; V3-23 vs 3.7 kb
- J κ differ in specificity potential: J κ 1/3 permissive

Acquired (somatic)

- Generation of CDR 3 during gene assembly: insertional residue (95a) determines affinity
- Hypermutation: positive and negative impact
- Antigen (vaccine) selection

Idiotype Expression in Vaccinated Infants



Infant Vaccination Protocol

Hib PS
Conjugate Vaccine



2 months

Hib PS
Conjugate Vaccine



4 months

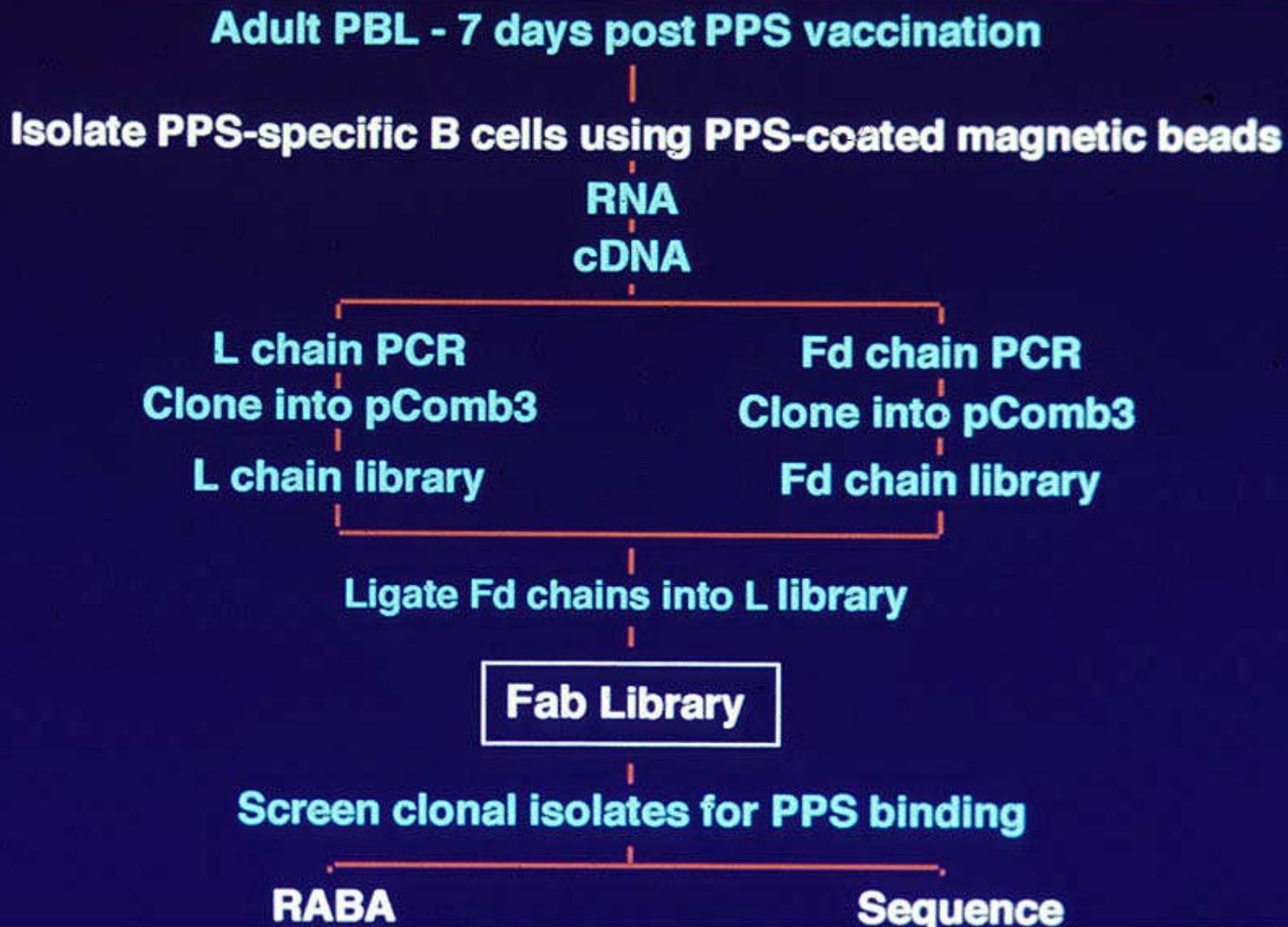


7 days → Blood

Repertoire Cloning ←



Cloning Human PPS-Specific Fab Fragments



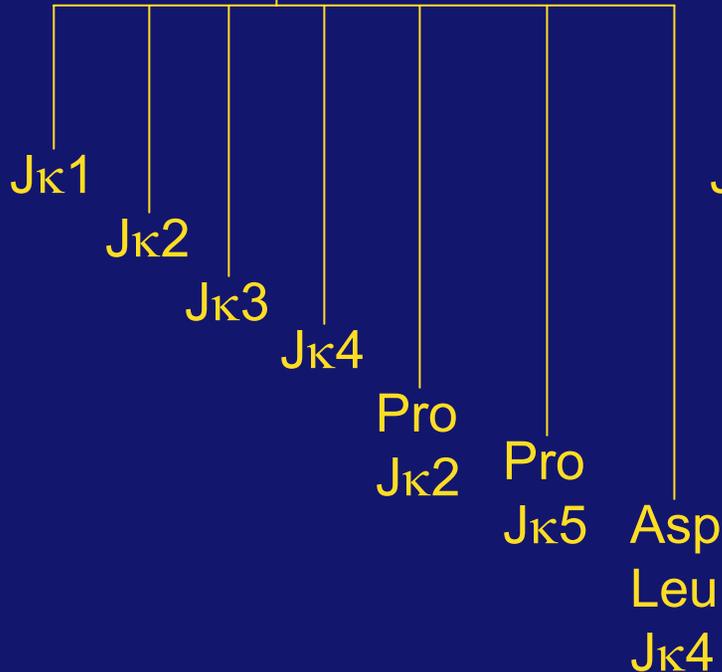
Anti-Hib PS VH & VL Rearrangements in Infants

Vaccine	Subject	VH	CDR3	JH	VL	95a	JK
OMPC	002	3-23	GYGMD	6b	A2a	R	1
					A2c	R	1
HbOC	008	3-23	GYGFD	4b	A2c	R	1
					A2c	L	1
					A2c	R	3
					A2c	P	3
OMPC	009	3-23	GYGFD	4b	A2a	R	1
					A2c	R	1
					A2c	R	3
OMPC	012	3-23	GYGFD	4b	A2c	R	1
OMPC	014	3-23	GYGMD	6b	A2c	R	1
					A2c	I	3
					A2c	L	3
					A2a	P	1
HbOC	015	3-23	GYGMD	6b	A2a	R	1
		3-23	GYGFD	4b	A2c	R	1
					A2c	R	1
OMPC	016	3-23	GYGMD	6b	A2a	R	1
					A2a	L	3
					A2c	R	1

Expression of A2 Light Chains in Fetal Liver and Cord Blood

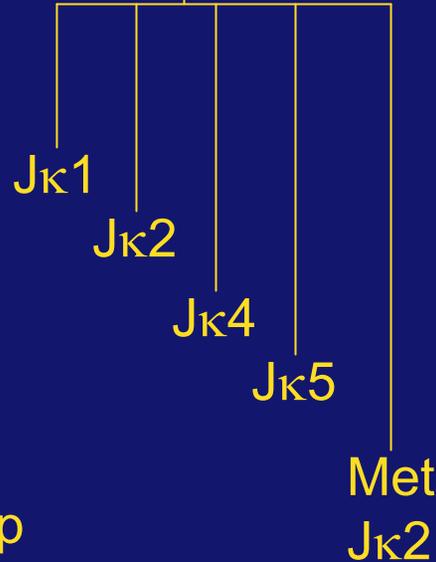
FL109

A2



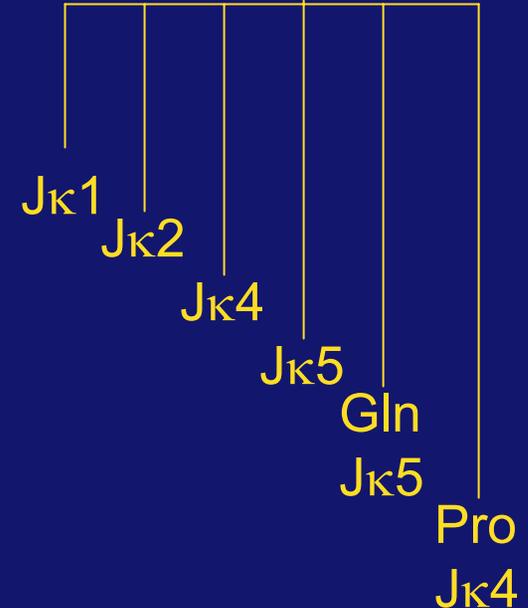
CB7

A2



CB13

A2



Ontogeny of HIB PS Repertoire

- Expression of canonical V configuration established by 2 months of age.
- Variation in L chain junctional residue (95a), in Jk usage and V3-23 allele usage can account for affinity variation amongst infant canonical antibodies.
- A2 V gene rearrangements, with and without junctional diversity are present in fetal liver (early 2nd trimester) and at birth.
- VH gene expression in neonates resembles that of adults. Mortari et al. J. Immunol. 150:1349, 1993; Bauer et al. J. Immunol. 169:1349, 2002.
- The Hib PS V repertoire is likely functional in the neonate.

Adult Anti-Capsular Antibody Responses

Pneumococcal PS

	Hib PS	6B	14	23F
Oligoclonal	yes	yes	yes	yes
Class-Switched	yes	yes	yes	yes
Canonical V	yes	no	?	yes
Affinity/Efficacy	yes	yes	?	yes
Mutation	yes (non-canon) no (canon)	yes	yes	yes